(54) **Conductivity measuring system comparing two detection channels**

(57) The system comprises two detection channels 11, 12, the first of which receives a mobile phase plus the analyte and the second of which receives only the mobile phase. Each channel has a detector 11, 12 comprising a microelectrode and a reference electrode for immersion in the received solution. An ac modulated voltage is applied across each pair of electrodes and the resulting current measured. Phase sensitive detection means 20 are provided for determining the faradic component of the current flowing between each pair of electrodes. The system further comprises means 21 for differentially combining the faradic components of the two channels and amplifier 22 for balancing the channels when mobile phase only is applied to both channels.
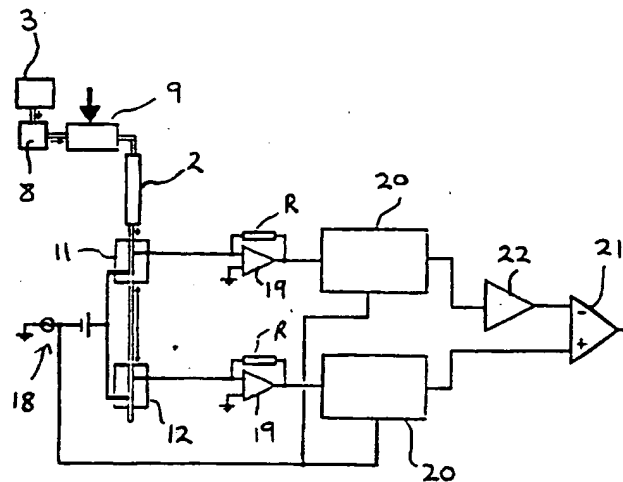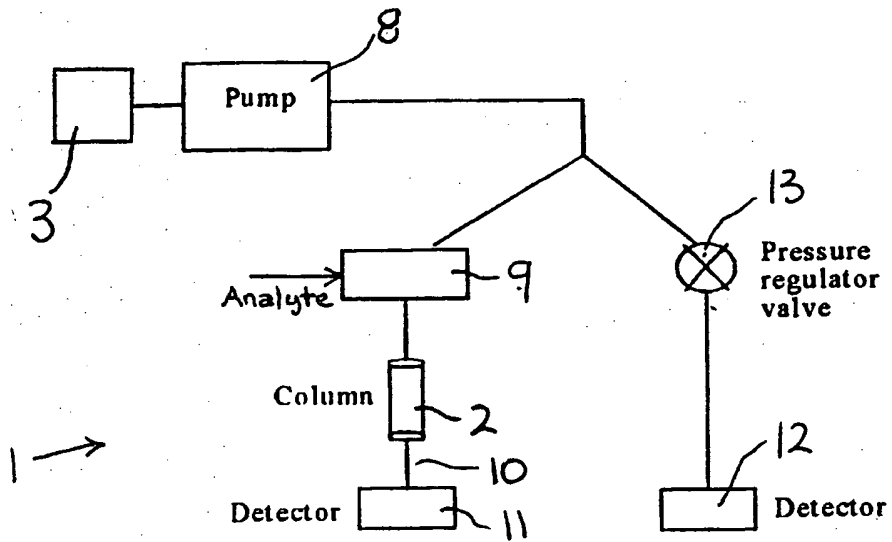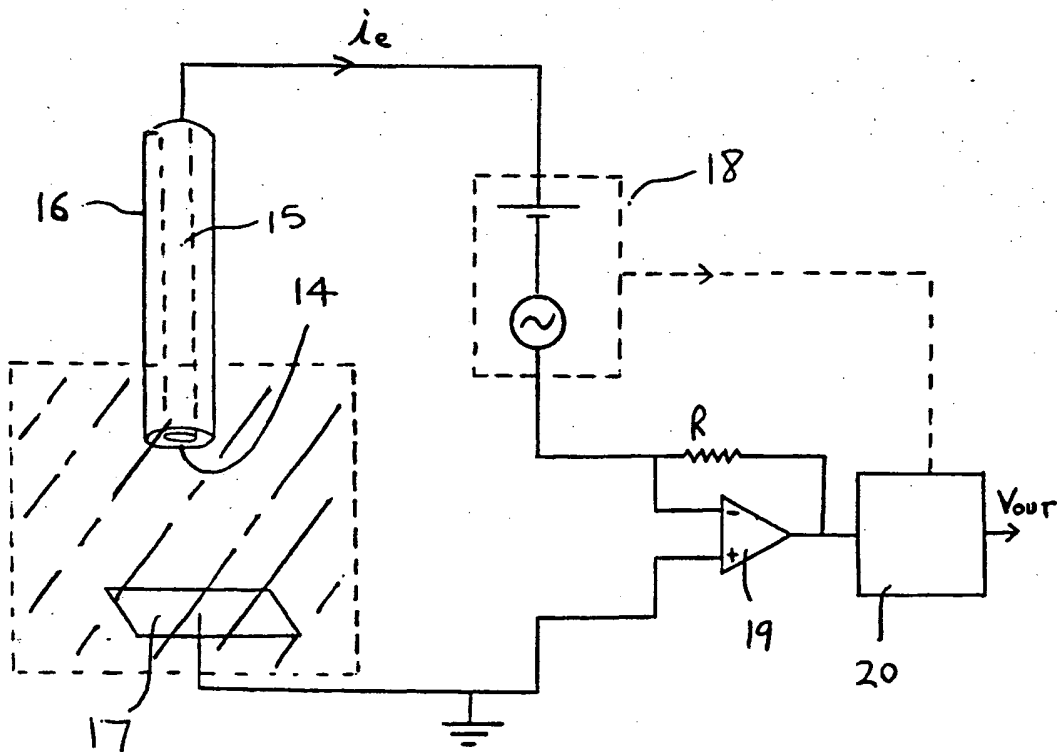
FIGURE 6

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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

EDS OUTPUT

(1)   (2)   (3)

t_1 t_2 t_3 t_4 TIME

FIGURE 5

3

9

8

2

R

11

20

19

R

20

22

21

18

12

19
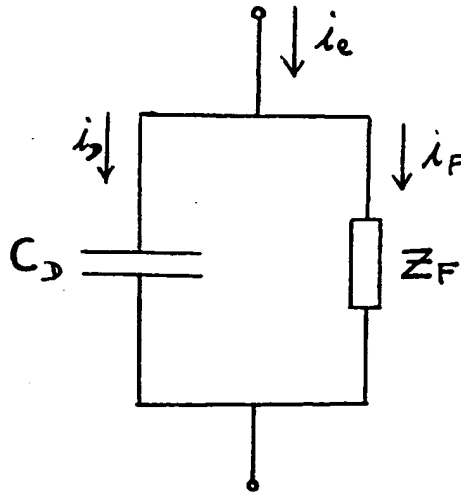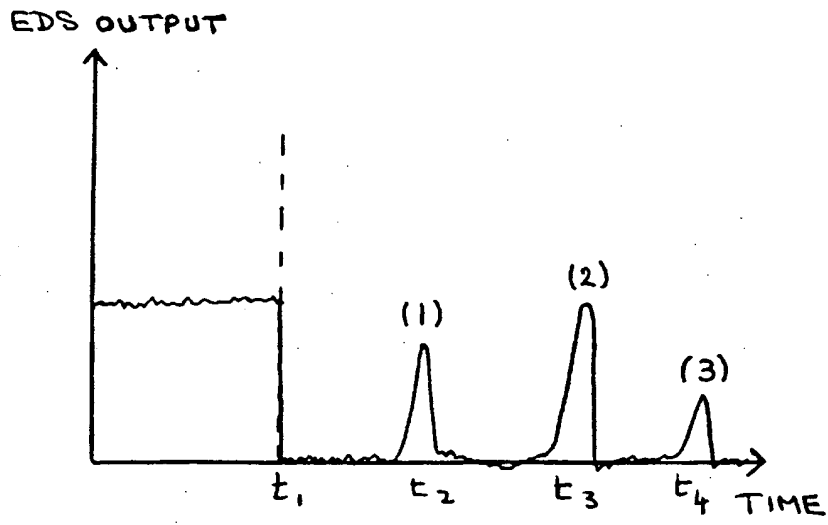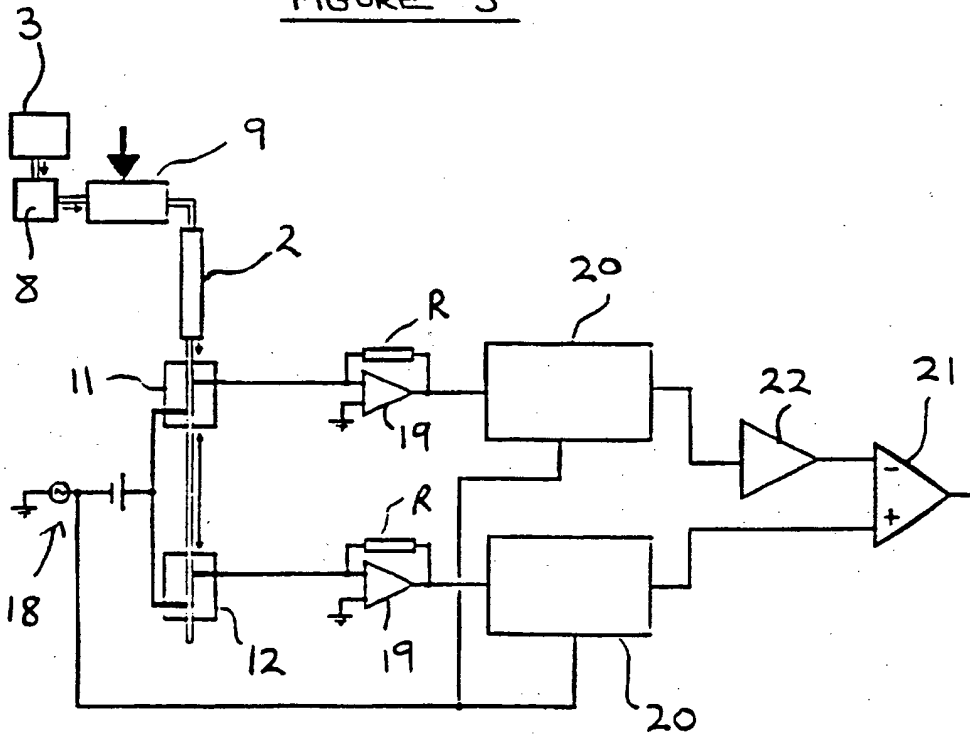
FIGURE 6

ELECTROCHEMICAL DETECTION SYSTEMS

The present invention relates to electrochemical detection systems and in particular, though not necessarily, to electrochemical detection systems for use in high performance liquid chromatography.

5   The use of amperometric sensors to determine the concentration of an ionised analyte present in a solution is widespread. Such sensors rely upon the dependence of faradaic current across an electrode/electrolyte boundary on the concentration of ions in the electrolyte. As the

10   ionic concentration of the electrolyte increases, for a given voltage applied between a pair of electrodes, the faradaic current will tend to increase. In order to estimate amperometrically the concentration of a specific analyte in a solution however, it is generally necessary

15   to first purify the solution to a point where it contains substantially only the analyte under investigation.

A commonly used purification technique is that known as high performance liquid chromatography (HPLC) which involves passing an unpurified solution, containing the

20   analyte of interest, under pressure through a column packed with very fine polymer beads (for example beads sold under the trade name "Sophadex"). The rat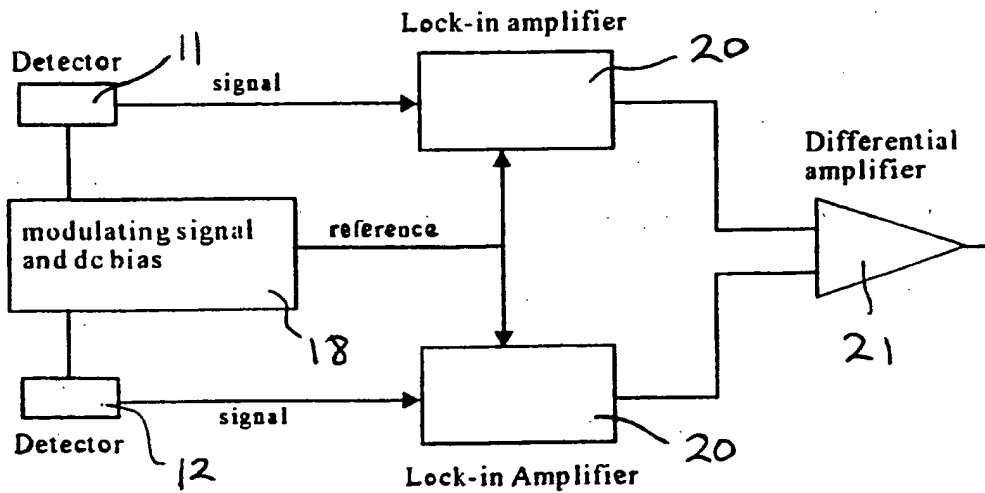e at which particular components of the unpurified solution flow through the column depends upon the size of the component and the

25   relative porosity of the column filling. Components having different sizes will flow through the column at different rates and the output from the foot of the column will be

a series of fractions containing different ones of the solution components. HPLC systems may be further refined by adding for example positive or negative charges to the beads to inhibit or advance the flow of certain components

5 through the column. In general, HPLC systems require to be calibrated by running various 'pure' samples through the column to determine the flow rate of specific components.

Amperometric sensing techniques are used to estimate the concentration of an analyte of interest contained in

10 a sample purified using HPLC. Whilst this technique has proved useful, conventional electrochemical detection systems offer a relatively limited detection range due primarily to noise. Noise arises due to a number of factors including electromagnetic interference and

15 contributions to the electrode/electrolyte current by phenomenon other than the faradaic effect. Additional problems with conventional electrochemical detection systems include the relatively long time required to establish a steady state current across the

20 electrode/electrolyte interface and the relatively low resistivity support solutions which must be used in order to maintain adequately high currents (to ensure a sufficiently high signal to noise ratio). In addition, the manufacturer of HPLC systems with integrated

25 electrochemical detection systems is difficult due to the relatively large size of existing electrochemical detection systems.

It is an object of the present invention to overcome

or at least mitigate certain of the disadvantages of conventional electrochemical detection systems. In particular, it is an object of the present invention to provide a low noise, highly sensitive microelectrode electrochemical detection system which can be integrated into an HPLC system.

According to a first aspect of the present invention there is provided an electrochemical detection system for use in determining the concentration of an analyte in solution, the system comprising at least two detection channels, each channel comprising:

first and second electrodes for immersion in a solution;

means for applying a voltage across said electrodes to cause a current to flow therebetween when the electrodes are immersed;

means for monitoring the flow of current between the electrodes when said voltage is applied; and

phase sensitive detection means for determining the faradic component of a current flowing between the electrodes,

wherein the electrodes of a first of the channels are arranged to be immersed in said solution in the absence of the analyte and the electrodes of the second channel are arranged to be immersed in said sample solution containing the analyte, the system further comprising means for differentially combining the faradic components of the two channels.

The provision of an additional reference channel and a differential output enable background noise common to both channels, e.g. electromagnetic interference or electrode currents resulting from the conductivity or electroactivity of the mobile phase (i.e. the solution in which the analyte is dissolved), to be substantially reduced. The use of phase sensitive detection means additionally enables the effect of electrode double layer capacitance to be substantially reduced.

Preferably for each channel at least one of the two electrodes is a microelectrode having a surface area of less than $0.01mm^2$ and preferably less than $0.0025mm^2$. The use of microelectrodes enables a reduction in the current flowing across the electrode/electrolyte boundary which in turn enables the use of mobile phases, in which the analyte is dissolved, having higher resistivities. The use of microelectrodes also reduces the time required to establish a steady state current across the electrode electrolyte interface.

Preferably, the means for applying a bias voltage across the two electrodes of each channel comprises means for applying a dc bias voltage, modulated with a relatively low voltage ac signal, across the electrodes. The bias voltage means may be arranged to operate in a pulsed amperometric detection mode. Preferably, the same voltage is applied across both electrodes from a common voltage source.

Preferably, the phase sensitive detection means of

each channel comprises a lock-in amplifier which receives as its reference signal the electrode a.c. bias voltage. The gain of the lock-in amplifier of one or both channels may be adjusted during a set-up stage, during which both channels receive only the mobile phase, to null the output of the differential combining means. Alternatively, a null setting may be achieved by incorporating a separate variable gain amplifier into one of the channels and varying the gain of that amplifier during the set-up stage.

Preferably, the differential combining means comprises a differential amplifier which provides at its output a signal proportional to the difference between the outputs of the two lock-in amplifiers.

According to a second aspect of the present invention there is provided an HPLC system for determining the concentration of a component of a sample solution, the system comprising an electrochemical detection system according to the above first aspect of the invention.

For a better understanding of the present invention and in order to show how the same may be carried into effect an embodiment of the invention will now be described with reference to the accompanying drawings, in which:

Figure 1 illustrates schematically a high performance liquid chromatography system incorporating an electrochemical detection system embodying the present invention;

Figure 2 shows an electrochemical detector of the system of Figure 1;

Figure 3 illustrates an equivalent circuit for an electrode/electrolyte interface;

Figure 4 shows schematically a circuit for implementing the electrochemical detection system of Figure

5   1;

Figure 5 illustrates a typical output signal from the circuit of Figure 4;

Figure 6 shows schematically an alternative embodiment of the present invention.

10   There is shown in Figure 1 a high performance liquid chromatography (HPLC) system 1 having a column 2 which is filled with an appropriate flow retarding filler as described above.   A mobile phase reservoir 3 contains a mobile phase supply, which can be water or another solvent.

15   The reservoir is coupled to the column 2 via a pump 8 and an injection valve 9 which maintain the required high pressure within the column.   The analyte is injected into the mobile phase at the injection valve 9.   The output 10 from the foot of the column is supplied to a detector 11

20   (EDS) which will be described hereinbelow.

The pump output is also coupled to a second detector 12 via a pressure regulator valve 13.   In operation, during an initial set-up stage the reservoir 3 supplies only the mobile phase which is in turn supplied to the two detectors

25   via respective valves (the detectors being at a lower pressure than the column).   Subsequently, the valve feeding the mobile phase to the second detector remains open and the analyte is injected into the column 2 via the injection

valve 9 to supply the mobile phase containing the injected analyte to the first detector.

Figure 2 shows in more detail the arrangement of the detectors 11,12 of the EDS of Figure 1 (both arrangements being substantially the same). The detectors comprise a microelectrode 14 which comprises the exposed end face of a platinum wire 15 (or other suitable material such as gold) extending through an insulating glass or plastic tube 16. Methods of producing such microelectrodes are well known. The detectors are also provided with a reference, or return, electrode 17 which has a relatively large surface area compared to the microelectrode (e.g. 10 to 100 times). The reference electrode may be of any suitable material although silver/silver chloride electrodes are preferred due to their relatively low impendence and their high electrical stability. Both the microelectrode and the reference electrode are arranged to be immersed in the solution fed via the pump 8.

The electrodes of each detector are coupled to a voltage source 18 which is arranged to apply both a dc bias voltage and a small ac modulating voltage in parallel across each pair of electrodes. Coupled in series between the electrodes of each detector and the voltage source 18 is an electrometer operational amplifier 19, having a feedback resistor r_f, which develops an output voltage proportional to the current i_e flowing between the microelectrode and the return electrode. The electrometer operational amplifiers present a very low impedance to the

respective circuits and therefore do not significantly load these circuits. A lock-in amplifier 20, to be described hereinbelow, is connected across each of the electrometer operational amplifiers 19. Preferably, pulsed amperometric detection is used in which the voltage is applied across the electrodes only in short pulses. This helps prevent fouling of the electrodes.

For each detector, the reference electrode 17 and the microelectrode 14 present two electrode/electrolyte interfaces across which current flowing around the circuit must pass. Both of these interfaces represent complex impedances in the series circuit although, as impedance is approximately inversely proportional to the interface surface area, for the purpose of analysis the impedance of the reference electrode 17 can be neglected. Figure 3 shows an equivalent circuit of the electrode/electrolyte interface presented by the microelectrode 14. The microelectrode interface can be represented as a capacitance C_D corresponding to the electrode/electrolyte double layer in parallel with a complex impedance Z_F representing the faradic contribution. Current flowing across the electrode/electrolyte boundary i_t will therefore comprise a first fraction i_D which flows through the double layer capacitance and a second fraction i_F which flows through the faradic impedance. It is this second fraction which is analyte concentration dependent and which must be derived in order to accurately estimate analyte concentration.

From the equivalent circuit shown in Figure 3, it is apparent that the double layer current will be phase shifted by approximately 90° whilst the faradic current will be shifted by somewhere between 0° and 90°, typically

5   45°. In order to separate out the faradic component, the voltage developed by the electrometer operational amplifier 19 is coupled as a measured signal to a lock-in amplifier 20 which also receives as a reference signal the ac modulating voltage from the voltage source 18. The lock-in

10   amplifier provides at its output a signal V_{out}, where

$$V_{out} = \frac{signal\ voltage \times ref\ voltage}{2} \cos(\theta + \phi)$$

where θ is the phase difference between the two signals and ϕ is an arbitrary phase shift. When $\phi = 0$, $\cos \theta$ will equal $1/\sqrt{2}$ when $\theta = 45°$ and will equal 0 when $\theta = 90°$. The lock-in amplifier therefore effectively nulls the double

15   layer current component i_D and provides an output which is substantially proportional to the faradic current component i_F. Variations in the surface properties of electrodes may cause the double layer and faradaic currents to be phase shifted, e.g. to 45° and 22° respectively. These shifts

20   can be compensated for by adjusting the value of ϕ which can be set in the lock-in amplifier.

Figure 4 shows a circuit arrangement for processing the outputs provided by the sample and reference detectors to provide a signal indicative of the concentration of a

25   component in the solution. As described above, both

detectors are fed by a common voltage source 18 which also provides the modulated bias voltage to the lock-in amplifier of each detector.

5   The compensated output signals are coupled to respective inputs of a differential amplifier 21 which provides an output signal proportional to the difference between the two compensated signals. Assuming that the electrodes, and other conditions, of both detectors are identical the effects of noise common to both detectors

10   will be eliminated.

In practice it is difficult or even impossible to obtain a perfect match between the detectors, e.g. due to manufacturing tolerances. However such differences can be compensated for by carrying out a set-up stage in which

15   both detectors receive only the mobile phase. The gain of the output stage of one of the lock-in amplifiers 20 is then adjusted to null the output of the differential amplifier 21.

Figure 5 illustrates a typical output of the system

20   of Figures 1. During the set-up stage, when the detectors receive only the mobile phase, the output of the differential amplifier is a substantially constant dc voltage indicating intrinsic differences between the two detectors. At a time t_1, the gain of one of the lock-in

25   amplifiers is adjusted to null the output of the differential amplifier. Subsequently the analyte, which contains at least three components, is introduced into the HPLC column. The component which travels fastest (1)

through the column produces a peak in the differential amplifier output at time t_2 whilst the slower travelling components (2) and (3) produce peaks at times t_3 and t_4 respectively. By precalibrating the detectors with standard solutions containing ones of the three components, the concentration of the components in the sample can be estimated from the amplitude of the peaks.

Figure 6 shows schematically an alternative embodiment of the present invention in which components already described with reference to Figures 1,2 and 4 are indicated with like reference numerals.

Rather than coupling the mobile phase pump 8 to the second detector 12 via a pressure regulator valve (as shown in Figure 1), this embodiment has both detectors 11,12 connected to the outlet of the separating column 2 with approximately 2m of tubing separating the two detectors. This length is such that by the time the analyte reaches the second detector 12 it will have passed through the first detector 11. Similarly, as the analyte passes through the first detector 11, it will not yet have reached the second detector 12. Thus the analyte will produce a pair of spikes of opposite phase at the output of the differential amplifier 21.

The embodiment of Figure 6 is further modified by the inclusion of a variable gain amplifier 22 coupled between the output of one of the lock-in amplifiers 20 and the differential amplifier 21. This eliminates the need for the lock-in amplifiers to have provision for varying their

gain and instead the gain of the amplifier 22 can be adjusted to null the output of the system.

It has been found that noise levels can be reduced significantly by shielding the electrode connections with

5   a shield driven by the modulated bias voltage.

It will be appreciated that variations may be made to the above described embodiment without departing from the scope of the invention. For example, instead of applying a continuous or pulsed voltage across the electrodes, a

10   saw-wave voltage may be applied to enable cyclic voltammetry to be carried out. The ac modulating voltage may be, for example, a sinusoidal or a square wave voltage. An embodiment of the invention may comprise more than two detection channels with switching means for coupling

15   selected ones of the channels to the solution reservoir.

CLAIMS

1. An electrochemical detection system for use in determining the concentration of an analyte in solution, the system comprising at least two detection channels, each

5   channel comprising:

first and second electrodes for immersion in a solution;

means for applying a voltage across said electrodes to cause a current to flow therebetween when the electrodes

10   are immersed;

means for monitoring the flow of current between the electrodes when said voltage is applied; and

phase sensitive detection means for determining the faradic component of a current flowing between the

15   electrodes,

wherein the electrodes of a first of the channels are arranged to be immersed in said solution in the absence of the analyte and the electrodes of the second channel are arranged to be immersed in said sample solution containing

20   the analyte, the system further comprising means for differentially combining the faradic components of the two channels.

2. A system according to claim 1, wherein at least one of the two electrodes is a microelectrode having a surface

25   area of less than $0.0025mm^2$.

3. A system according to claim 1 or 2, wherein the means for applying a bias voltage across the two electrodes of each channel comprises means for applying a dc bias

voltage, modulated with a low voltage ac signal, across the electrodes.

4. A system according to claim 3, wherein the same voltage is applied across both electrode pairs from a common voltage source.

5. A system according to claim 3 or 4, wherein the phase sensitive detection means of each channel comprises a lock-in amplifier which receives as its reference signal the corresponding electrode a.c. bias voltage.

6. A system according to claim 5, wherein the gain of the lock-in amplifier of one or both channels may be adjusted during a set-up stage, during which both channels receive only the mobile phase, to null the output of the differential combining means.

7. A system according to claim 5 and comprising a variable gain amplifier coupled in series with one of the lock-in amplifiers so that a null setting may be achieved during a set-up stage, during which both channels receive only the mobile phase, to null the output of the differential combining means.

8. A system according to any one of the preceding claims, wherein the differential combining means comprises a differential amplifier arranged to provide at its output a signal proportional to the difference between the outputs of the two lock-in amplifiers.

9. An electrochemical detection system substantially as hereinbefore described with reference to Figures 1 to 5 of the accompanying drawings or with reference to those

Figures as modified by Figure 6.

10. An HPLC system for determining the concentration of a component of a sample solution, the system comprising an electrochemical detection system according to any one of

5   the preceding claims.

11. An HPLC system substantially as hereinbefore described with reference to Figures 1 to 5 of the accompanying drawings or with reference to those Figures as modified by Figure 6.

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Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.N): G1N NBCC, NBMF, NBMK, NBMX.

Int Cl (Ed.6): G01N 27/06, 27/08, 27/10;   G01R 27/22.

Other: ONLINE: WPI.

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
Y	GB 1,585,067	NRDC	1, 8, 10.
Y	GB 1,398,947	HARTMANN & BRAUN	1, 8, 10.
Y	EP 0,018,419 A1	EISAI	1.
Y	US 5,180,968	NEW YORK STATE UNIVERSITY	1, 8, 10.
Y	US 5,138,264	HITACHI	1, 8, 10.
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